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Evidence of increased histamine levels in lung lavage fluids from patients with cryptogenic fibrosing alveolitis

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SUMMARY

In this study we report a significant increase in histamine in lung lavage fluids from a group of 33 patients with lone cryptogenic fibrosing alveolitis (lone CFA), and from a group of 13 patients having CFA in association with other connective tissue disorders, when compared with findings for 13 smoking patient controls without peripheral lung disease ($P<0{\cdot}001$, $P<0{\cdot}05$ respectively). The increases were independent of smoking or treatment. Significant correlations were obtained between the raised histamine levels in CFA and increased levels of albumin and increased counts of neutrophils and eosinophils in the lavage fluids, and with more pronounced fibrosis in CFA lung biopsies. Thus histamine is associated with features of inflammation relating to progressive or more severe disease. No significant increase in histamine was observed in a group of 22 patients with sarcoidosis, although 21 had evidence of disease involving the lung parenchyma. There were, however, significantly higher levels in the patients with X-ray evidence of upper lobe contraction, suggestive of 'fibrosis' ($P<0{\cdot}025$). The levels also showed a correlation with increasing counts of lavage neutrophils ($P<0{\cdot}005$), a feature also associated with X-ray evidence of contraction in this group. Mast cells were readily identified in biopsies from 12 CFA patients suggesting that these cells may provide one possible source of histamine in CFA lungs. These observations raise the question whether histamine, and/or possibly other substances derived from mast cells, plays any role in amplifying inflammation associated with pulmonary fibrosis.

INTRODUCTION

Recent studies of broncho-alveolar lavage fluids have greatly advanced our understanding of the mechanisms of inflammation in interstitial lung disorders, and some of the information is already proving of clinical value (Haslam *et al.*, 1980). We now report increased levels of histamine in lavage fluids from patients with cryptogenic fibrosing alveolitis (CFA). This evidence suggests that vasoactive amines may contribute to the local inflammatory events in this non-atopic group of patients.

PATIENTS AND METHODS

Patients. We have studied samples of the broncho-alveolar lavage fluid from 46 patients with cryptogenic fibrosing alveolitis (CFA). Thirty-three of these patients had only the lung disorder (lone CFA), while 13 also had associated connective tissue disorders of other systems (CFA + CT).

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To provide comparison with an inflammatory pulmonary disease of a different kind, we have also studied a group of 22 patients with sarcoidosis. One patient was at radiographic stage 1 with only bilateral hilar lymphadenopathy, but the remaining 21 all had evidence of disease involving the lung parenchyma, eight at stage II with parenchymal as well as hilar shadows, and 13 at stage III with only parenchymal shadows. Seven of those with parenchymal disease also had evidence of upper lobe contraction or linear shadows suggestive of fibrosis. As controls we included a group of 13 smoking patients without evidence of parenchymal lung disease or chronic bronchitis. These patients were undergoing fibreoptic bronchoscopy for investigation of suspected bronchial carcinoma, subsequently confirmed in seven of the cases. Lavage was performed in a bronchoscopically and radiographically normal area of the lung. The diagnostic criteria for case selection have been described previously (Haslam *et al.*, 1980; Mitchell *et al.*, 1977). Details of patient ages, smoking and treatment are summarized in Table 1.

Methods. The lavage procedure was performed as previously described (Haslam *et al.*, 1980). Details of lavage fluid introduction and recovery volumes and percentage fluid recoveries are given in Table 2.

Lavage fluid samples were freed from cells by centrifugation at 300 ***g*** for 10 min, and 2-ml aliquots of cell-free fluid were acidified and deproteinated using perchloric acid at a final concentration of 6% then stored at −70°C pending assay for histamine. Histamine was assayed using a modification of the automated spectrofluorometric technique as described by Siraganian (1975). Estimates of albumin and IgG in the cell-free fluids made from radial immunoassay studies employing Behring antisera, and estimates of soluble immune complexes from C1q-binding studies were used for correlative purposes, but will be reported in detail elsewhere. Differential counts of cells from the fluids (Haslam *et al.*, 1980) were also available for correlations. Lung biopsy specimens fixed in glutaraldehyde and processed through osmium tetroxide for electron microscopy (EM) were available from 12 CFA patients, including 11 who had undergone lavage. Counts of nucleated cells with metachromatic cytoplasmic granules were made using 1-μm-thick sections stained with toluidine blue at the level of the light microscope (× 100 objective) over a standard area of 1 mm^2. Transmission EM was used to check the identity of the cells as mast cells or basophils. Electron microscopy preparations of cells from lavage fluids were also examined for the presence of mast cells or basophils.

Statistical methods used for data analysis are indicated in Figs 1 & 2 and Tables 2–5.

Table 1. Details of patients

Diagnosis	No. of patients			Mean age ± s.d. (years)	Age range (years)	Treatment at lavage	
	Male	Female	Total			Untreated	On treatment
Lone CFA							
Smokers	22	4	26	55±9	33–69	20	6
Non-smokers	4	3	7	50±14	36–79	4	3
Total	26	7	33	55±10	33–79	24	9
CFA + CT							
Smokers	5	0	5	50±10	40–62	2	3
Non-smokers	3	5	8	47±10	32–64	3	5
Total	8	5	13	50±10	32–64	5	8
Sarcoidosis							
Smokers	9	6	15	37±13	25–64	12	3
Non-smokers	4	3	7	33±8	23–43	7	0
Total	13	9	22	36±12	23–64	19	3
Smoking controls	10	3	13	49±16	22–65	13	0

Table 2. Lavage fluid introduction and recovery volumes and percentage fluid recoveries

		Lavage fluid volumes						Lavage fluid albumin levels		
		Introduction volume (ml)		Recovery volume (ml)		Per cent fluid recovery				
Diagnosis	No. of patients	Mean*	s.d.	Mean*	s.d.	Mean*	s.d.	No. of patients	Median value (mg/100 ml)	Absolute range (mg/100 ml)
Lone CFA	33	399	70	114	26	29	8	30	9·8†	3–22·3
CFA + CT	13	429	90	118	30	28	9	12	10·4†	5·3–29·7
Sarcoidosis	22	360	81	134	26	39	11	22	10·5†‡	4·5–40·0
Smoking controls	13	374	61	117	36	33	10	13	6·8	4·4–19·6

* No significant differences compared with smoking controls using unpaired Student's *t*-test for parametric data.

† Significant increase compared with smoking controls using Mann–Whitney *U*-test for non-parametric data.

‡ No significant difference compared with lone CFA or CFA + CT using Mann–Whitney *U*-test.

RESULTS

The results of lavage fluid histamine assays are shown in Fig. 1. Histamine levels were significantly increased compared with smoker controls in lone CFA ($P < 0{\cdot}001$) and in CFA + CT ($P < 0{\cdot}05$). No significant differences of histamine levels were demonstrated comparing smokers and non-smokers with CFA, nor with those untreated at lavage compared with those undergoing treatment.

There was no significant increase in lavage fluid histamine levels for the group of patients with sarcoidosis compared with the smoker controls (Fig. 1).

The histamine levels showed no general correlation with lavage fluid introduction or recovery volumes or percentage fluid recoveries (using Spearman rank correlation coefficients), and these did not differ significantly between the groups (Table 2); neither did the histamine levels reflect the content of serum components in the fluids as monitored by fluid albumin levels which were comparably increased compared with smoker controls in sarcoidosis as well as in CFA (Table 2). Erythrocytes which might indicate trauma during lavage were either absent or present in very small numbers in the fluids, with only two exceptions.

Correlations made to examine whether the local increases in histamine in CFA might reflect local increases in capillary permeability and the influx of any of the different inflammatory cells found in the lungs of these patients are shown in Table 3. A highly significant quantitative association was obtained between the histamine levels and the levels of albumin in the fluids ($P < 0{\cdot}005$), albumin being used as a marker for serum exudation into the fluid. A similarly close correlation was obtained with the levels of IgG in the fluids ($P < 0{\cdot}005$).

Of the three types of inflammatory cells which have been studied in lavage fluids from CFA patients, there was a significant association of increasing lavage histamine levels with increasing counts of neutrophils ($P < 0{\cdot}01$) and eosinophils ($P < 0{\cdot}025$), but not of lymphocytes. There was no correlation between levels of lavage histamine and the levels of soluble immune complexes in the fluids as detected using the C1q-binding method.

Comparisons within the sarcoidosis group demonstrated that, although histamine was not significantly increased compared with controls, patients with X-ray evidence of upper lobe contraction as well as parenchymal involvement had significantly higher lavage histamine levels than those with parenchymal involvement without contraction ($P < 0{\cdot}025$; Fig. 2). In addition, the only significant correlation between histamine levels and other lavage features was with increasing counts of lavage neutrophils ($P < 0{\cdot}005$; Table 4), a feature which is also associated with X-ray evidence of contraction (Arnoux *et al.*, 1980).

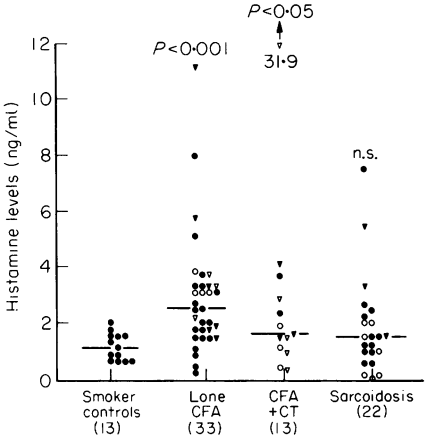

Fig. 1

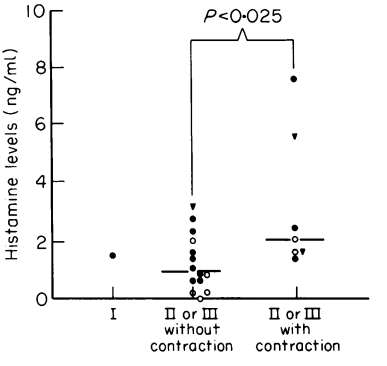

Fig. 2

Fig. 1. Histamine levels in lavage fluids from patients with interstitial lung disorders. (o) Untreated non-smoker, (•) untreated smoker, (▽) treated non-smoker, (▼) treated smoker. (——) Median values; P = level of significance by comparison with smoker controls using Mann-Whitney U-test; n.s. = not significant.

Fig. 2. Relationship of increasing lavage histamine with X-ray evidence of linear opacities and/or upper lobe contraction suggestive of 'fibrosis' in sarcoidosis. I = hilar involvement only, II = parenchymal and hilar involvement (eight cases), III = parenchymal involvement (13 cases). (o) Untreated non-smoker, (•) untreated smoker, (▼) treated smoker. (——) Median values; P = level of significance using Mann–Whitney U-test.

Table 3. Lavage histamine level correlations in CFA

Correlation with:	No. of CFs	Spearman rank correlation r	Spearman rank correlation P (1-tailed)
Lavage			
Albumin levels	41	+0·51	<0·005
IgG levels	41	+0·49	<0·005
% neutrophils	41	+0·38	<0·01
% eosinophils	41	+0·33	<0·025
% lymphocytes	41	−0·18	n.s.
% C1q binding	41	−0·09	n.s.
Biopsy			
Mast cell counts	11	+0·39	n.s.
Fibrotic field counts	11	+0·53	<0·05

Counts made to establish the presence of mast cells or basophils in lung biopsies from 12 CFA patients as possible sources of local histamine are shown in Table 5. Cells confirmed to have the transmission EM features of mast cells (Fig. 3) were readily demonstrable in the interstitial tissues (mean 27 cells per 1 mm^2 area), and were significantly more common in thickened fibrotic than in thin alveolar septa ($P < 0·025$). Higher power observation of the mast cell granules revealed not only typical scroll forms, but also frequent particulate forms of granule described by Kawanami *et al.* (1979) as suggestive of partial degranulation (Fig. 3b). The EM studies could not demonstrate either mast cells or basophils, except in very rare instances, in the air spaces of the CFA lung biopsies or amongst cells from the CFA lavage fluids. There was no significant association of the biopsy mast cell counts with the levels of histamine in the lung lavage fluids in 11 CFA cases where information

Table 4. Lavage histamine correlations in sarcoidosis

Correlation with:	No. of CFs	Spearman rank correlation	
		r	*P* (1-tailed)
Lavage			
Albumin levels	22	+0·02	n.s.
IgG levels	22	+0·09	n.s.
% lymphocytes	22	−0·20	n.s.
% neutrophils	22	+0·60	<0·005

Table 5. Mast cell counts in CFA lung biopsies (12 patients)

	Number of nucleated cells per mm^2	Per cent distributed in:	
		Thin-walled fields	Fibrotic fields
Mean	27	36	64
s.e.m.	6	8	8
Absolute range	2–73	0–82	18–100
		$P<0·025$*	

* Mann–Whitney *U*-test.

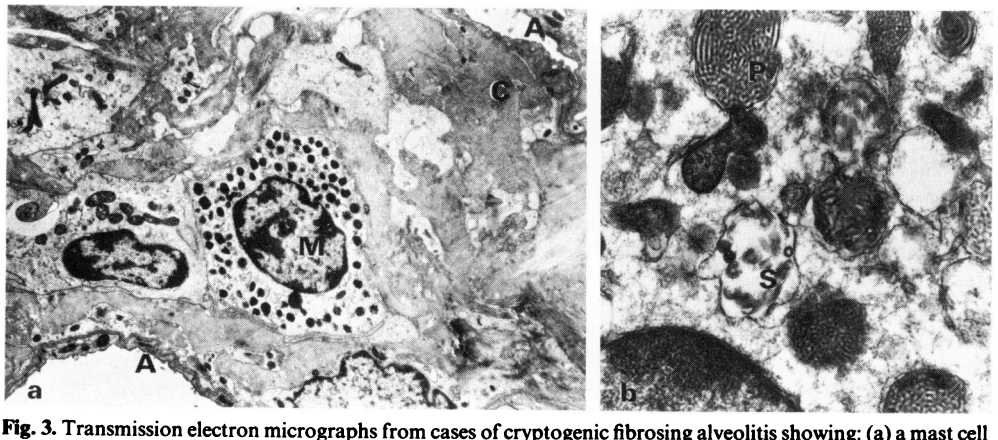

Fig. 3. Transmission electron micrographs from cases of cryptogenic fibrosing alveolitis showing: (a) a mast cell (M) in a thickened alveolar septum. A = alveolar space, C = collagen. (× 2,650). (b) Higher power view of mast cell granules reveals not only the typical scroll forms (S), but also a particulate form of granule (P) described by Kawanami *et al.* (1979) as suggestive of partial degranulation. (× 17,750).

on both was available, but interestingly the higher histamine levels tended to occur in those patients whose biopsies had shown higher proportions of thickened fibrotic alveolar septa ($P < 0{\cdot}05$; Table 3).

DISCUSSION

This study has demonstrated the presence of histamine in the lungs of patients with cryptogenic fibrosing alveolitis and has shown that this is associated with certain other components of inflammation in this disorder which relate to progressive and more severe disease. Whether these occur as part of the primary events in the lung or develop as a consequence of secondary amplification circuits is not established. In either case, this now raises the question whether histamine or other mast cell products play any part in the continuing inflammatory processes found in this disorder.

Leitch & Kay (1979) have reported that histamine is increased in sputum and saliva samples from smoking compared with non-smoking normal volunteers, but differences in smoking could not provide an explanation for the lavage fluid histamine increases in CFA which were similar in smoking and in non-smoking patients. Nor could the histamine increases be explained as any general consequence of variability in fluid introduction of recovery volumes or percentage fluid recoveries, or of content of serum due to trauma or exudation.

In view of this, the observed close correlation of increasing lavage fluid histamine levels with albumin levels in our CFA patients suggests that events associated with histamine release are also associated with the degree of increased vascular permeability in the lungs of these patients. The further association observed between higher lavage histamine levels and more extensive fibrosis in CFA lung biopsies also indicates that lavage histamine increases in CFA are an unfavourable feature. In view of this, new therapeutic approaches aimed at inhibiting vasoactive amine release or function might be worth consideration in this serious lung disorder.

Correlations observed between histamine increases and increasing counts of neutrophils and eosinophils, but not of lymphocytes, in the CFA lavage fluids suggest that inflammatory events associated with release of histamine may also contain chemotactic stimuli for granulocytes. The lack of association with lymphocytes suggests that the influx of these cells may be under the control of a different inflammatory circuit. This possible distinction was also indicated by our earlier finding of a significant association of lavage lymphocyte increases with corticosteroid response in CFA, but conversely of lavage eosinophil and neutrophil increases with failure to respond (Haslam *et al.*, 1980; Rudd *et al.*, 1980). Thus the observed correlation of lavage histamine increases in CFA with increased granulocyte counts provides another observation linking histamine with an apparently unfavourable inflammatory circuit in CFA. Although histamine was not significantly increased for the total patients with sarcoidosis, the slightly higher levels in the patients with X-ray evidence of upper lobe contraction are of particular interest since they suggest that this may also indicate an unfavourable course towards fibrosis in sarcoidosis. In this respect, the relationship of histamine with lavage neutrophils but not lymphocytes in sarcoidosis as well as CFA is also of interest, particularly since this is another feature which shows an association with X-ray evidence of contraction in this disorder (Arnoux *et al.*, 1980; Haslam *et al.*, unpublished observations).

The presence of mast cells in reasonably large numbers in our CFA lung biopsies with frequent particulate granule forms suggestive of partial degranulation (Kawanami *et al.*, 1979) implies that these cells could be one possible source of histamine in CFA lungs. The mast cell counts obtained are of a similar order to counts recently reported by Kawanami *et al.* (1979) in their patients with idiopathic pulmonary fibrosis (IPF). These workers also reported a significant increase in the mast cell counts in IPF by comparison with non-fibrotic controls. Interest in a possible association of increased mast cell numbers with fibrosis has previously arisen from time to time (Selye, 1965), but this is the first study to provide evidence that release of mast cell products may be occurring in CFA lungs. Mast cell products such as histamine and other vasoconstrictors released together with vasodilators, such as prostaglandin E_1, might not only provide one possible explanation for increased vascular permeability in CFA, but other products such as chemotactic factors for

eosinophils and neutrophils could also explain the observed association of histamine increases with increased counts of these granulocytes. We have no information on agents interacting with mast cells in CFA lungs. This might involve cytophilic IgE or IgG antibodies or perhaps local activation of complement. Such activation could result in the generation of fragments of C3 and C5 (C3a and C5a fragments) with anaphylatoxic properties. Substances reported in CFA lavage fluids which might activate or interact with the complement system generating anaphylatoxic fragments are soluble immune complexes (Gadek *et al.*, 1979a) and collagenase (Gadek *et al.*, 1979b, Johnson, Ohlsson & Olsson, 1975). Soluble immune complexes detected by C1q binding in our CFA lavage fluids did not, however, show any correlation with increases in lavage fluid histamine, and so far there is no information regarding complement-derived anaphylatoxins in CFA lungs.

Whatever the explanation for the local histamine increases in CFA, the importance of these observations is that they identify one means by which damage to the vascular capillaries might occur to allow influx of inflammatory cells into the interstitial tissues and air spaces of the lungs.

The therapeutic implications of these observations perhaps in contribution with other treatments should now be considered.

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